Pharmacology Biochemistry & Behavior, Vol. 22, pp. 357–360, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

Modifications in Adrenal Hormones Response to Ethanol by Prior Ethanol Dependence

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Received 27 March 1984

GUAZA, C. AND S. BORRELL. *Modifications in adrenal hormones response to ethanol by prior ethanol dependence.* PHARMACOL BIOCHEM BEHAV 22(3) 357–360, 1985.—Ethanol was administered to rats by means of a liquid diet for 16 days; after an ethanol-free interval of four weeks, animals received a test (IP) dose of ethanol (2 g/kg), and the adrenocortical and adrenomedullary responses were evaluated. Chronically ethanol-exposed animals showed tolerance to the stimulatory effect of ethanol in the pituitary-adrenal axis. Likewise, previously dependent rats showed tolerance to the increase in the activity of the adrenomedullary function induced by acute administration of the drug. Our results indicate that chronic ethanol ingestion can induce persistent changes after complete alcohol abstinence.

Ethanol dependence Corticosterone Dopamine Norepinephrine Epinephrine

CHRONIC intoxication with ethanol has been shown to result in tolerance to and physical dependence on this drug [7,15]. The possibility has been considered that tolerance to some actions of addictive drugs persists for some time after abstinence [10]. Several authors have reported that morphine addiction leads to physiological modifications that are observable for a long period of time after the withdrawal syndrome [4, 9, 16]. In the case of alcohol, long-term tolerance effects have also been reported [8,15] and there is evidence that animals may develop a potentiation of withdrawal symptomatology if they have previously been dependent on ethanol [2, 3, 27].

Chronic ethanol treatment results in tolerance to the stimulatory action of the drug on the pituitary adrenal axis [13, 14, 17, 23]. The development of tolerance to ethanol effects on adrenomedullary function has not been clearly determined. We previously observed that in the adrenal medulla the response to a test dose of ethanol was modified during chronic ethanol ingestion [11].

The purpose of the present study was to investigate whether prior ethanol dependence could alter the response of the adrenomedullary and/or adrenocortical functions to an ethanol challenge dose, after a period of time of recovery (ethanol-free).

METHOD

Male Wistar rats (IFA, CREDO, France) initially weighing 180–200 g, were used. The rats were housed two to a cage, for ethanol ingestion, in a ventilated room at a temperature of about 24°C and with a 12-hour light-dark schedule.

The animals were placed on an all-liquid diet consisting of chocolate-flavored Meritene (Wander, Spain), with 35% or 38% of the total calories represented either by added ethanol or by an isocaloric volume of sucrose solution. In addition, liquid diets were supplemented with vitamins (Vitamin Diet Fortification Mixture, ICN Pharmaceuticals, Inc., Cleveland, OH). In order to adapt the animals to liquid diet ingestion, they were placed on a preliminary 4-day liquid diet of sucrose; after this time elapsed, the sucrose was substituted with the appropriate amounts of ethanol to render a diet with 35% of the total calories represented by ethanol. After 3 days of this diet, the proportion of ethanol was increased to 38% of the total caloric values; this diet was maintained for 13 days. Each 2 rats/cage was provided with 160 ml/day of the liquid diet. Equal volumes of the diet were given to both the ethanol and the sucrose animals.

The response to a test dose of ethanol in chronic ethanol-treated rats was studied by giving an IP dose of 2 g/kg of ethanol four weeks after cessation of alcohol or sucrose ingestion. Additional groups of control animals on laboratory chow were injeced with the same dose of ethanol or saline. The animals were killed 1 hr after injection. In order to eliminate cyrcadian variations, animals were killed between the hours of 10:30 and 11:30 a.m. The rats were killed by decapitation with the aid of a guillotine; the blood was collected from the trunk and was allowed to clot for two hours. The blood was then centrifuged and the serum collected and frozen at −20°C until assays were performed. The adrenal glands of each rat were rapidly removed, weighed, and further cut into two equal halves. The individual halves were weighed and a left adrenal half was pooled with a right adrenal half for determination of the content of adrenal corticosterone; the

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TABLE 1

ADRENOCORTICAL RESPONSE TO A TEST DOSE OF ETHANOL (2 g/kg, IP) 4 WEEKS AFTER CESSATION CHRONIC ETHANOL INGESTION

Pretreatments	Corticosterone	
	Adrenal Gland (μg/g)	Serum (μg/100 ml)
Lab Chow plus saline	24.39 ± 3.74	15.87 ± 2.08
Lab Chow plus 2 g/kg ethanol	59.76 ± 8.62*	58.98 ± 1.38*
Sucrose plus 2 g/kg ethanol	64.56 ± 4.58*	60.24 ± 4.35*
Chronic ethanol plus 2 g/kg ethanol	24.46 ± 6.55‡	25.49 ± 6.09†‡

Results are the means ± S.E.M. from 7–18 rats per group.
*$p<0.001$; †$p<0.05$ vs. lab chow plus saline.
‡$p<0.001$ vs. sucrose or lab chow plus ethanol.

two remaining adrenal halves were pooled for catecholamine measurement. The procedure above mentioned was run while keeping the tissue in petri dishes maintained on ice, until homogenization. Adrenal and serum corticosterone were measured fluorimetrically according to the procedure described by Matsumura *et al.* [20].

Adrenal glands were homogenized in 0.4 N perchloric acid prior to purification and concentration of the amines by absortion in activated alumina according to the method described by Shellenberger and Gordon [25]. For the simultaneous fluorescence assay of dopamine, norepinephrine and epinephrine, we used, slightly modified as previously described [11], the hydroxyindole method [21].

An Aminco-Bowman spectrophotofluorometer was used. Statistical analysis of the data was performed using analysis of variance (ANOVA). When the overall ANOVA was significant, an a posteriori Student-Newman-Keuls test was applied to study the differences among the means.

RESULTS

The administration of ethanol by means of a liquid diet resulted in a mean daily ethanol intake of 15 g/kg. Daily observations revealed signs of intoxication, including docility and decreased locomotor activity. The removal of ethanol after 16 days of consumption resulted in the appearance of a variety of withdrawal symptoms such as tremors, tail stiffening, and irritability; in parallel experiments, we observed that 90% of ethanol-treated rats exhibited audiogenic convulsions when they were exposed to 500 cps sound at 110 dB of intensity for 45 sec. Sucrose-treated animals did not show convulsions at any of the sound frequencies studied.

Mean body weights (±SEM) at the beginning and at the end of the chronic experiments were 192±5.89 and 226±4.79 g for the sucrose group; 193±7.53 and 225±4.01 g for the chronic ethanol group. Mean body weights (±SEM) prior to the test dose of alcohol were 320±7.01 g for the sucrose group; 304±6.93 g for the ethanol chronic group and 312±3.53 g for the lab chow group. The animals on the lab chow and injected with saline weighed 254±2.90 g. No significant differences in the adrenal glands weights among the four experimental groups of animals were found (data are not shown).

Adrenocortical Function

Table 1 shows the data obtained on serum and adrenal corticosterone levels. The ANOVA of the data revealed a significant influence of treatments on serum corticosterone, $F(3,40)=52.12$, $p<0.001$, and on adrenal hormone concentration, $F(3,29)=27.04$, $p<0.001$. The administration of a test dose of ethanol to lab chow or sucrose treated rats induced significant increases in serum and adrenal levels of corticosterone in comparison with control animals (lab chow rats injected with saline). Chronic ethanol-treated rats showed serum and adrenal levels of corticosterone significantly lower than sucrose or lab chow-treated animals in response to a test dose of alcohol. Serum hormone levels were slightly, but significantly higher in chronically treated rats subjected to ethanol challenge than in the lab chow animals injected with saline. However no significant differences in adrenal levels of corticosterone were found.

Adrenomedullary Function

Adrenal levels of dopamine, norepinephrine and epinephrine are presented in Table 2. ANOVA of the data revealed significant effects of treatments on levels of dopamine, $F(3,31)=9.91$, $p<0.001$. No significant effects were found in norepinephrine and epinephrine concentrations. The injection of 2 g/kg of ethanol to lab chow or sucrose treated rats produced significant increases in adrenal levels of dopamine as regards to control animals (lab chow rats injected with saline). Chronic ethanol-treated rats challenged with a test dose of ethanol showed levels of dopamine significantly lower than sucrose liquid diet or lab chow-fed animals also subjected to a test dose of the drug. No significant differences were found in the adrenal content of dopamine between chronic ethanol-treated rats in response to alcohol injection and lab chow animals injected with saline.

DISCUSSION

The schedule we used for ethanol administration in a liquid diet induced physical dependence, as demonstrated in a previous work [12]. This is corroborated by the fact that 90%

TABLE 2

INFLUENCE OF PREVIOUS CHRONIC ETHANOL INGESTION IN THE ADRENAL VARIATIONS OF DOPAMINE, NOREPINEPHRINE AND EPINEPHRINE INDUCED BY A TEST DOSE OF ETHANOL (2 g/kg, IP) ADMINISTERED TO RATS 4 WEEKS AFTER CESSATION ETHANOL INTAKE

Pretreatments	Dopamine (μg/g)	Norepinephrine (μg/g)	Epinephrine (μg/g)
Lab Chow plus saline	5.0 ± 0.53	156.5 ± 12.84	834.8 ± 30.07
Lab Chow plus 2 g/kg ethanol	10.1 ± 0.75*	148.0 ± 12.96	954.0 ± 50.24
Sucrose plus 2 g/kg ethanol	10.6 ± 1.52†	183.0 ± 17.66	835.9 ± 82.08
Chronic ethanol plus 2 g/kg ethanol	5.9 ± 0.61‡	199.7 ± 20.70	878.4 ± 46.50

Results are the means ± S.E.M. from 7–9 rats per group.
*$p<0.001$; †$p<0.01$ vs. lab chow plus saline.
‡$p<0.02$ vs. sucrose or lab chow plus ethanol.

of ethanol chronic-treated rats presented audiogenic convulsions during ethanol withdrawal.

It is known that acute ethanol administration stimulates the pituitary-adrenal axis, inducing increases in serum corticosterone levels [13, 17, 23]. The development of tolerance to this initial stimulatory effect has also been described [13, 14, 17]. The results of the present study showed that serum and adrenal levels of corticosterone after ethanol challenge were significantly lower in rats chronically ingesting ethanol and undergoing a 4-weeks ethanol free period than in the corresponding sucrose-fed animals. Kakihana *et al.* [14] have shown in mice, that adrenocortical response to ethanol was significantly lower in chronically treated animals than in controls; in the above mentioned study two weeks after ethanol cessation, chronically treated mice had a diminished response to acute ethanol administration; however, four weeks of abstinence was sufficient to restore the normal responsiveness in plasma corticosterone levels to acute ethanol. Those authors [14] indicated that alcohol metabolism, adrenal sensitivity and corticosterone turnover are not likely to be the main responsible factors for the differences observed in ethanol-adapted and non-adapted mice.

As regard to adrenomedullary function, an overactivity of the system has been described after acute ethanol treatment [1, 5, 22]. The adrenal levels of dopamine have been considered as indicators of adrenomedullary hormone biosynthesis [26]. In this way, we previously found [11] that, after an injection of 2 g/kg of ethanol to normal rats, levels of adrenal dopamine were greatly increased. In the present study, one month after ethanol deprivation, rats previously addicted to ethanol showed, in response to a test dose of ethanol, dopamine levels significantly lower than those observed in sucrose or lab-chow-fed rats also injected with ethanol.

The present data suggest that chronic ethanol administration may result in a long-term alteration in physiological mechanisms or biochemical pathways, modified primarily by the drug. Although we cannot discount the possibility of an alteration in ethanol metabolism, this explanation seemed unlikely four weeks after ethanol exposure. Ritzman and Tabakoff [24] demonstrated in mice that, 24 hr after ethanol withdrawal, animals injected with a test dose of ethanol showed no differences in blood ethanol levels in comparison to sucrose-fed mice, but were found to be tolerant to the effects of alcohol on rectal temperature and sleep time.

It has been shown that the disruptive effect of a test dose of ethanol on REM sleep was greater 6–8 months after ethanol cessation in ethanol-experienced rats than in ethanol-naive animals. Also, rats in the second ethanol administration sequence acquired tolerance more rapidly than in the first ethanol exposure [15,19]. In addition, an hypothesis of potentiation of ethanol withdrawal by prior physical dependence on ethanol has been derived from the results of several studies [2, 3, 6, 18].

It seems clear from the above-mentioned studies and from our results that ethanol, when administered chronically, can induce persistent changes, as reflected by the tolerance to a challenge dose of ethanol after complete ethanol abstinence. These findings raise serious questions about the reversibility of the pharmacological effects of this most broadly used addictive drug.

ACKNOWLEDGEMENTS

The authors wish to express their appreciation to A. Ortiz for her expert technical assistance. This work has been supported by CAICYT grants Nos. 42030, 2122.

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